THE CHROMOSOME NUMBER AND KARYOTYPE OF SENECIO ISATIDEUS DC.

By E. P. Goldsmith and S. Krupko.

(With Plates XIII and XIV.)

1. Introduction.

Almost a century after the species had originally been described (de Candolle, 1837), Steyn (1931) included *Senecio isatideus* DC. in a list of plants that he had proved toxic and that had not previously been recorded as poisonous. This plant and the alkaloids isolated from it have been studied chiefly from toxicological and chemical points of view. de Waal (1939) reported that the species has isatidine as the main alkaloid with lesser amounts of retrorsine. He investigated the hydrogenation, hydrolysis and chemical structure of isatidine (de Waal, 1940) and also the chemical structure of its necine and necic acid (de Waal 1941 *b* and 1941 *c*). The toxicity and pathological systems produced by the species and its alkaloids have been investigated by Steyn (1934), Steyn and van der Walt (1941) and Harris, Anderson and Chen (1942).

An intensive search through the literature brought to light no reference to the cytology of the species. It was hoped, therefore, that the present study would help to fill this gap in our knowledge.

2. Methods and Materials Used.

I. METHODS USED IN STUDYING THE CHROMOSOMES AT MEIOSIS.

The following statement recently made by Darlington and La Cour (1942) gives some indication of the great importance modern cytologists attach to smear and squash preparations. " Sections have now been largely replaced by smears and squashes for all but the smallest masses of material." As is well-known, the advantages of these methods are rapidity of fixation, rapidity of handling without embedding and integrity of the nucleus.

For convenience, the first and second meiotic divisions will be referred to simply as I and II.

(a) *Material.*

The plants selected for study were found growing along the left bank of the stream below Murray's dam at Frankenwald, the Botanical Research

Station of the University of the Witwatersrand, near Johannesburg. Before the beginning of the 1945–46 growing season, rhizomes were transplanted from here to a flower bed near the Biology Block in the University grounds at Milner Park, Johannesburg. The plants which grew from these rhizomes were healthy and sturdy, and squash preparations of the pollen mother cells were made from the anthers of the floret buds of the young capitula produced in November and December, 1945. Specimens from both Frankenwald and Milner Park pressed later in the season and taken to the National Herbarium, Pretoria, were identified as *Senecio isatideus* DC. (See Plate XIII.)

(b) *Temporary preparations.*

The aceto-carmine squash technique (Schneider, 1880 ; Heitz, 1926) was used with intermittent heating (McClintock, 1929). The anthers were mounted and squashed singly. The anther was so small that the cover slip came into direct contact with the pollen mother cells, making it unnecessary to remove the anther walls or any other debris. The edges of the cover slip were sealed (McClintock, 1929) and drawings were done immediately.

The most troublesome part of the procedure was to find pollen mother cells at the metaphase stage. The stage of nuclear division could not be correlated with the external appearance of any part of the plant, and there was no alternative but to select at random the anthers for squashing. The fact that so few metaphases were encountered for so many late prophases and so many anaphases indicated that the intervening metaphase must be of short duration. Not only were the anthers in a single floret at widely different stages, but sometimes the pollen mother cells of a single anther would show all stages from early prophase I to telophase II. Often days at a time would pass in which not a single metaphase was seen, in spite of observations showing that meiosis proceeded actively from 6 a.m. throughout the day till 9 p.m. (No observations were carried out from 9 p.m. to 6 a.m.). All attempts to see the first post-meiotic divison were unsuccessful.

Three modifications of the technique were tried with the following unsatisfactory results :—

(1) Squashing the anther and removing the larger fragments of debris before placing the cover slip in position involved damaging and losing a large proportion of the pollen mother cells.

(2) Keeping the anther in a variation of Carnoy's fixative—4 volumes of absolute alcohol to 1 volume of glacial acetic acid—for 24 hours prior to squashing (McClintock, 1929) did not improve the staining sufficiently to justify including this modification in the routine procedure.

(3) Smear methods could not be used as the anthers were small and not sufficiently succulent.

(c) *Permanent preparations.*

The same technique for squashing was used as for the temporary preparations. The cover slip was fastened into position on the slide with a brass paper fastener as suggested by Müller (1945). Held together in this way, the slide and cover slip were passed through a series of acetic acid—95% alcohol solutions (McClintock, 1929), then into dioxan ; and finally the preparation was mounted in dioxan balsam. Dioxan made by J. T. Baker Chemical Co., Phillipsburg, N.J., was used with good results.

(d) *Microscope and drawing apparatus.*

The optical system used to study and draw the chromosomes consisted of a 15 × eyepiece, an achromatic, 97 × (1 ·88 mm.), 1 ·25 N.A. oil immersion objective (Bausch & Lomb) and a 1 ·25 N.A. Abbé condenser. The total magnification obtained with this eyepiece and objective was 1455 ×. Drawings were done at table level with the aid of a camera lucida, giving a magnification of 2800 ×.

II. METHODS USED IN STUDYING THE CHROMOSOMES AT MITOSIS.

Sections of the root tips were cut in order to obtain evidence as a check, and to supplement the observations made, on the squash preparations of the pollen mother cells.

Haematoxylin was selected as the stain most likely to remain intense permanently.

(a) *Material.*

Early one morning, a few days after the first heavy spring rains of the 1945–46 growing season, rhizomes were dug up below Murray's dam. The soil was removed by dipping the plants in the stream and the tips of the season's new roots were fixed in the way described on pp. 9 and 10 The plants were taken to the laboratory during the time the roots were soaking.

Various methods were tried to induce the production of new roots from rhizomes transplanted to flower pots or kept, both with and without Spergon, between damp sheets of blotting paper in an oven at 35° C. The following cutting treatments were tried :—

(1) No cutting treatment.

(2) Roots all cut off about an inch from the rhizome.

(3) Roots all cut off a few millimetres from the rhizome.

(4) Half the roots on the rhizome cut as for (2), the other half receiving no cutting treatment.

(5) Half the roots on the rhizome cut as for (3), the other half receiving no cutting treatment.

Each of these cutting treatments of the roots was combined in turn with each of the following soaking treatments at room temperature of the roots and their cut ends immediately after cutting :—

(1) No soaking treatment.

(2) Soaking in tap water for 6 hours.

(3) Soaking in tap water for 4 hours and then in Hortomone A solution of " the normal strength " for 16 hours.

(4) Soaking in tap water for 4 hours and then in Hortomone A solution of " half the normal strength " for 16 hours.

The Hortomone A solutions were made up as directed in the literature accompanying the preparation, which is distributed by Plant Protection, Ltd., Yalding, Kent.

Each of the above cutting and soaking treatments of the roots was combined in turn with both the following treatments of the leaves :—

(1) No cutting treatment.

(2) Leaf blades all entirely cut off.

The plants grown in flower pots were dug up at weekly intervals and, after careful removal of the soil in a gentle trickle of tap water, inspected for root development. The plants kept in damp blotting paper at 35° C. were inspected daily. Those which had been treated with Hortomone A were soaked once a fortnight for a further 16 hours in a freshly made-up solution of the same " strength " as in the original treatment received, before replacing in the flower pots or in the oven.

None of the treatments induced the production of more than a few extremely slender new branches arising from the old roots, even after they had been continued for several months.

(b) *Fixation.*

The following fixatives were used as described :—

A.—Modifications of Navashin Fixative.

(1) *S. Navashin fixative* (M. Navashin, 1934).

The following formula was used :—

1 ml. glacial acetic acid
4 ml. 16% formaldehyde
10 ml. 1% chromic acid.

Using tap water for the soaking treatments, root tips were fixed under the following conditions :—

(*a*) in the field (protecting the fixative from direct sunlight) ;

(*b*) after soaking in water at room temperature for 2, for 3, for 4 and 6 hours ;

(*c*) after keeping at 2° C. without water for 6 or 14 hours, and then soaking in water at room temperature for 15 minutes ;

(*d*) after soaking in water at 2° C. for 6 or 14 hours, and then in water at room temperature for 15 minutes.

(2) *Stockholm fixative* (Maheshwari, 1939).

The following formula was used for solution A :—

1 gm. chromic acid
10 ml. glacial acetic acid
65 ml. distilled water.

The following formula was used for solution B :—

40 ml. 40% formaldehyde
35 ml. distilled water.

After soaking in tap water at room temperature for 3 hours, root tips were fixed in equal volumes of solutions A and B freshly mixed together.

(3) *Taylor's modified Karpetchenko fixative* (Johansen, 1940).

The following formula was used :—

1·5 ml. 10% chromic acid
10 ml. 10% acetic acid
0·83 ml. 40% formaldehyde
23·67 ml. distilled water.

Root tips were fixed after soaking in tap water at room temperature for 2 hours.

B.—Modifications of Lewitsky Fixative (Avdulow, 1931).

As Lewitsky's (1931) original publication was unobtainable in South Africa, the formulae given by Avdulow (1931) were followed, trying both possible interpretations for his " 10% formalin."

Root tips were fixed in each of four modifications of Lewitsky fixative after soaking in tap water at room temperature for 1 hour.

In addition, root tips were fixed in Lewitsky fixative for big chromosomes with 4% formaldehyde after soaking in tap water at 2° C. for 14 hours and then in tap water at room temperature for 15 minutes.

The root tips were gently boiled in freshly mixed fixative at room temperature for 10 minutes, using a water suction pump to reduce the pressure. Root tips fixed in the field were taken with the least possible delay to the laboratory, where they were boiled in the same way. Before washing, root tips fixed with modifications of Lewitsky fixative were kept in 1% chromic acid for 3 days (Navashin, 1934).

(c) *Staining.*

After bleaching, Heidenhain's well-known haematoxylin-iron-alum schedule for staining was followed. It was found necessary to bleach the sections before staining because of the presence of tannins. If the sections were not bleached, the chromosomes were obscured by darkly-staining globules of various sizes. When sections of fresh root tips were placed in 10% ferric chloride with a little sodium carbonate (Johansen, 1940), the globules turned a bluish-green colour, indicating the presence of tannins. The method of bleaching depended on the fixative used.

(1) After modifications of Navashin fixative, the slides were left in Stockwell's bleaching solution for 24 hours. The following formula for this solution was used :—

90 ml. distilled water
1 gm. potassium dichromate
10 ml. glacial acetic acid
1 gm. chromic acid.

According to Johansen (1940), the chromic acid renders the precipitated tannins soluble, the acetic acid removes them and the potassium dichromate acts as a catalyst in preparing the tissues for very sharp staining.

(2) After modifications of Lewitsky fixative, the slides were left in 1% chromic acid for 4 days. Stockwell's bleaching solution could not be used on account of the acetic acid it contains, but chromic acid by itself bleached the tannins sufficiently well.

After modifications of Lewitsky fixative the slides were left in 6% iron alum for 24 hours for mordanting before transferring to the haematoxylin solution.

(d) *Thickness of sections.*

After considering the results obtained with the usual preliminary sections, it was decided to cut transverse sections at 8 μ for counting the chromosomes and for comparing the effects of the different methods of fixation used, and at 7 μ for studying the morphology of the chromosomes. Only metaphases in the periblem and epidermis were examined and, before making any critical observations, the neighbouring sections on either side were inspected to make sure that no nuclei that had been damaged in cutting were studied.

3. Observations Made.

At metaphase I of meiosis, the chromosomes were present as the monoploid number of tetrads of which, whenever they were well spread out, there were invariably 10. As seen from the direction of the spindle pole, the chromosomes appeared more or less square in outline, and the four chromatids of the tetrad could frequently be distinguished (see Plate XIV, Fig. 10). Sometimes, however, only the bivalent structure was visible and even this was not always discernible. In these cases, there seemed to be no reason to doubt the actual presence of the tetrad and it was assumed that, by some coincidence of staining or due to the fact that the view obtained of the particular chromosome was not strictly polar, the tetrad structure was not obvious. The chromosomes differed from one another only slightly in size and shape and, as has usually been found in plants at this stage, the shapes of the individual chromosomes varied from one pollen mother cell to another, making it impossible, with perhaps a single exception, to recognise the chromosomes by their morphological peculiarities. In many pollen mother cells, a particular chromosome in the metaphase configuration was slightly turned away from the pole, revealing an elongated form characterised by two constrictions set at about even thirds along its length and a fairly pronounced tendency to be V-shaped (see Plate XIV, Figs. 4, 5, 10). It is very tentatively suggested that this chromosome may be identical either with chromosome IX or X or with both these chromosomes in the root tips (see below). In equatorial view, the chromosomes were more or less oblong in outline. In spite of the fact that no sort of pretreatment for structure was ever used, the coiled chromonemata were often observed. In some polar views of the metaphase plate, certain of the chromosomes were turned away from the pole and were seen in views intermediate between polar and equatorial. In the latter chromosomes, the spirals appeared as fine lines (see Figs. 6*a*, 6*c* and 10). In equatorial view the spirals showed as broad bands (see Figs. 7*a*, 7*c*). Anaphase I showed unfailing regularity.

In polar view, the chromosomes also appeared more or less square in outline at metaphase II and the two chromatids of the dyad were usually visible. Anaphase II showed unfailing regularity. Telophase II was immediately followed by cytokinesis and the pollen mother cell was divided simultaneously into four young pollen grains.

Whenever the chromosomes in the root tips lay well enough separated from one another to display their number, there were invariably 20 to be counted. The metaphase chromosomes in the root tips, as has usually been found in plants, did not show, with the one exception already referred to, and further discussed below, any marked resemblance to the chromosomes seen in the pollen mother cells. In general, they appeared very slender in comparison with the latter. After fixation with S. Navashin fixative in the field, the chromosomes were swollen to different degrees and varied in diameter from 0·30 to 0·60 μ with a mean of 0·45 μ. Although the chromosomes were interlaced to a large extent, it was possible to establish the identity of the 10 homologous pairs by combining the observations made on a few selected metaphase plates (see upper row of chromosomes Fig. 1). The chromosomes were only of medium size, yet they were well differentiated morphologically. Further, while the cytogenetic aspect of the matter was not investigated, it was noted that the morphology of each type varied only over the small range usual for plants not of complex hybrid origin. Individual chromosomes could, thus, be recognised by their characteristic forms in other metaphase configurations examined later. The morphological peculiarities of the chromosome complement are summarised in the following table which is amplified in the discussion below.

Pair.	*Approximate Shape.*	*Kinetochore.*	*Secondary Construction.*
I	C	Not obvious	One obvious
II	S	Distinct	Two obvious, one in each curve
III	L	Not obvious	Not obvious
IV	Rod	Distinct	Not obvious
V	V with arms unequal	Not obvious	Not obvious
VI	V with arms equal	Not obvious	Not obvious
VII	V with arms equal	Distinct	Not obvious
VIII	V with arms unequal	Distinct	One very conspicuous
IX	V with arms equal	Not obvious	Two obvious, one about the middle of the arm, the second in the other arm but slightly more towards the bend of the V than the middle of the arm
X	V with arms equal but usually slightly longer and of slightly smaller diameter than chromosome IX	Not obvious	Two obvious, one about the middle of each arm.

It must be pointed out that, in all cases, the remarks on the kinetochore, though probably valid, may be open to correction, since neither the detailed structure of the region in question nor the rôle it played at anaphase were investigated. Where no constrictions whatever were observed in the chromosomes, it was assumed that the position of the kinetochore would, without doubt, be revealed by methods of fixation specially adapted to the study of this organ rather than the general morphology of the chromosome. Where it is stated that, while secondary constrictions were visible, the kinetochore was not seen, it was concluded that the constrictions observed would be unlikely to function as the kinetochore. This conclusion was based on our knowledge of the position of the kinetochore definitely established for the great majority of chromosomes of a similar shape previously studied in other species.

The chromosome complement consisted of members of five distinct shapes, namely C, S, L, rod and V. One pair of each of the first four shapes was present, and no less than six pairs of V-shaped chromosomes. In the C-shaped type, the constriction was always located approximately where the top of a cedilla would come in a printed letter " C " (see upper row of chromosomes, Fig. 1). It may be noticed that the members of the pair drawn display a striking difference in diameter, but attention has already been called to the varying degrees of swelling shown by the chromosomes after fixation with S. Navashin fixative in the field. S is a shape rarely recorded for chromosomes in plants. It seems uncertain, however, whether it is really uncommon or just difficult to detect as, owing to its curved form, it may come to lie in a large number of different planes, its true morphology thus escaping recognition. In the present investigation the S chromosome was comparatively easy to see. The constrictions were set at about even fourths along the length of the chromosome and the middle one was taken to be the kinetochore. It is not for a minute suggested that the possession of a mere split end could, with any justification at our present state of knowledge, be regarded as the morphological peculiarity of a single type of metaphase chromosome, since all chromosomes are known to undergo a longitudinal splitting at this stage. However, it is surely noteworthy that, while none of the other chromosomes of the set ever displayed a visible double structure, the L-shaped type was always clearly seen to be split along about half its length, even after fixation with Stockholm fixative (see Plate XIV, Fig. 13) when only some of the morphological details were preserved. The rod-shaped type was characterised by a slight but definite swelling, starting a little to one side of the kinetochore and increasing gradually towards the end of the rod on the same side.

Three morphological characters served to distinguish the V-shaped chromosomes from one another, namely, the number of constrictions, the arm length ratio and the angle at which the arms were set. These distinguishing features are written in order of their constancy in the morphology of the chromosomes from which, of course, derived their relative importance as guides to classification. On the basis of the number of constrictions seen, the V-shaped chromosomes could be classified into three groups with two, one and three pairs of chromosomes respectively, as follows :—

A.—Chromosomes with no constrictions visible (chromosomes V and VI).

(1) Chromosome V had one arm about two-thirds the length of the other, with the angle of divergence up to 60°.

(2) Chromosome VI had both arms about equal in length and diverging at 90° or more. It may be remarked that the members of the pair drawn for chromosome VI display, as do the members of the pair drawn for chromosome I, a striking difference in diameter, but the reader is again referred to the measurements given on page 00 for confirmation that this is not due to a drawing error.

B.—Chromosomes with one constriction visible (chromosome VII).

(1) Chromosome VII had the solitary constriction, which was taken to be the kinetochore, situated in one arm very near the region of forking of the V, though never exactly in the fork. Both arms are about equal in length and diverge at an angle of 90° or more.

C.—Chromosomes with two constrictions visible (chromosomes VIII, IX and X).

Of all the chromosomes in the set, the individuals of the three types in this last group exhibited the widest variation in morphology. The angle at which the arms were set varied over such a wide range in each of the types that any figures given should be of little or no value.

(1) Chromosome VIII was easily distinguished from the other two types because, firstly, it had a more or less centrally-placed constriction which was taken to be the kinetochore and, secondly, although the arm length ratio varied so widely that a statement of its numerical limits would convey no meaning, the arms were usually unequal in length. The V was curved rather than pointed, and the variation in the position of the arc with alteration in the arm length ratio seemed to have no particular relation to the position of the kinetochore. Furthermore, chromosome VIII was unique, not only among the members of group C of the V-shaped chromosomes, but also among the members of the entire set, in the conspicuousness of its secondary constriction, which was several times as long as the kinetochore and located near the end of one of the arms. This constriction was probably associated with the organisation of the nucleolus.

(2) It might be concluded from the table and from Fig. 1, that chromosomes IX and X were almost identical but, strangely enough, these two types were seldom if ever confused when examining the preparations. Nevertheless, it cannot be denied that they resembled one

another closely and that both of them resembled, to some extent, the only chromosome which was identified as an individual at metaphase I of meiosis, namely the longish chromosome tending to be V-shaped and with two constrictions set at about even thirds along its length (see Plate XIV, Figs. 6*e*, 5*c* and 10). However, two such individuals were seen once only in one and the same metaphase (see Fig. 10) and further study would be needed to verify that the observations at meiosis had not been confined to a single individual but rather to two chromosomes with the same degree of similarity as chromosomes IX and X in the root tips, and to establish the identity of these chromosomes with chromosomes IX and X.

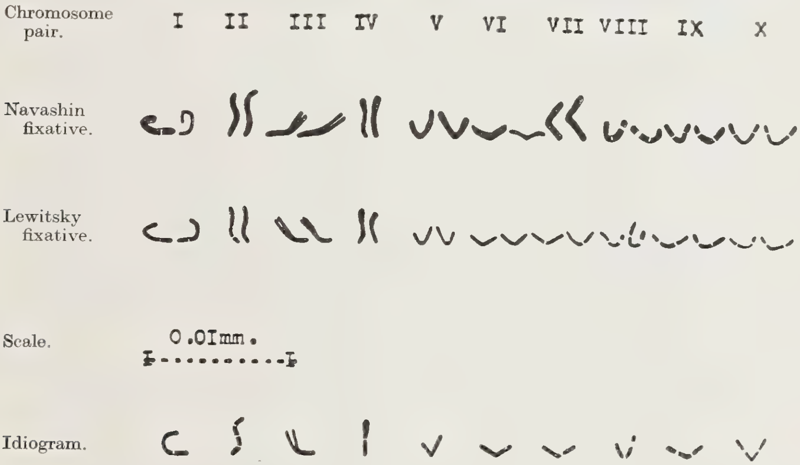

Fig. 1.
The karyotype drawn from root tip metaphases after fixation with S. Navashin fixative (upper row of chromosomes) and Lewitsky fixative for big chromosomes with 4 per cent. formaldehyde (middle row of chromosomes). Homologous pairs of chromosomes and scale—2130x. Idiogram approximately 2130x.

Soaking at room temperature for 2 hours prior to fixation decidedly improved the spreading of the chromosomes at the metaphase. Soaking for 3 hours led to a further marked improvement in the spreading. After soaking for 6 hours, however, the chromosomes were badly entangled and, in many cases, gave the impression that mitosis had stopped at late metaphase.

Cold treatment for 6 hours in the absence of water prior to fixation brought about a pathological condition in the tissues. The cells were plasmolysed, the stainability, especially of the nuclei, was increased and the chromosomes showed varying degrees of shrinkage. Deeply stained chondriomes were seen. After cold treatment for 14 hours in the absence of water, the pathology was further advanced and the chromosomes had coalesced into a shrunken mass.

Soaking at 2° C. for 6 hours shortened and straightened the chromosomes and improved their spreading (see Fig. 12). The members of the chromosome complement differed only slightly from one another in shape, the constrictions were not visible, and it was no longer possible to recognise the individual chromosomes by their morphological characters. Soaking at 2° C. for 14 hours led to a further improvement in the spreading with which, however, the chromosomes came to occupy several horizontal planes. The ordinary two dimensional drawing, therefore, does not reflect the real improvement made in the preparation. When soaking at 2° C. for 14 hours was followed by fixation with S. Navashin fixative, the chromosomes varied in diameter from 0·58 to 0·87 μ with a mean of 0·63 μ. Although the lower limits of the latter range overlapped slightly the upper limits of the range of diameters after fixation with S. Navashin fixative in the field, the mean diameter was greater by half nearly, and it might be concluded that soaking at 2° C. probably tended to increase the chromosome diameter. This increase was more clearly demonstrated by the use of Lewitsky fixative (see below).

After Stockholm fixative, the chromosomes were fairly uniformly swollen, varying in diameter from 0·45 to 0·75 μ with a mean of 0·60 μ. The chromosomes were well spread and some of the morphological details were preserved (see Fig. 13).

After Taylor's modified Karpetchenko fixative, the metaphase presented much the same appearance as after S. Navashin fixative.

After all four modifications of Lewitsky fixative, the chromosomes were thinner than after S. Navashin fixative and comparatively uniform in diameter, varying only from 0·25 to 0·35 μ with a mean of 0·30 μ. In spite of their thinness, however, the chromosomes were no better spread than they were after the latter fixative, nor were the constrictions noticeably easier to see. The best contrast between the staining of the chromosomes and cytoplasm was obtained after Lewitsky fixative for big chromosomes with 4 per cent. formaldehyde. As the percentage of the formaldehyde was gradually increased according to the different modifications of the formula, the contrast deteriorated steadily but, with careful differentiation, moderately good preparations could be made even after Lewitsky fixative for small chromosomes with 10 per cent. formaldehyde. Comparatively few mitochondria and leucoplasts were seen. The fact that after Lewitsky fixative for big chromosomes with 4 per cent.

formaldehyde the members of the chromosome complement showed essentially the same morphological characteristics as after S. Navashin fixative (see middle row of chromosomes, Fig. 1), confirmed the detailed observations on the peculiarities of the individual types described above. The use of a different fixative had little or no influence on the effects of preliminary soaking at 2° C. for 14 hours (see Fig 14). The chromosomes varied in diameter from $0 \cdot 5$ to $0 \cdot 8\ \mu$ with a mean of $0 \cdot 65\ \mu$, that is over the identical range and with almost exactly the same mean as when this pretreatment was followed by S. Navashin fixative (see p. 111). From an inspection of the corresponding ranges and means it is quite evident that soaking at 2° C. had doubled the diameter of the chromosomes. On account of the varying degrees of swelling shown by the chromosomes after fixation with S. Navashin fixative in the field (see p. 110), the real extent of the increase in chromosome diameter produced by soaking at 2° C. was somewhat obscured by the use of the latter fixative, only a tendency to the same effect being discernible.

After S. Navashin and Stockholm fixatives the nucleolus in the resting cells was grey, and after Taylor's modified Karpetchenko and Lewitsky fixatives it was dark blue.

4. Discussion of Observations.

The present account is the first record, to the best of the writer's knowledge, of any cytological investigation of a South African species of Senecio. Ishikawa (1916), Okabe (1931) and Matsuura and Sutô (1935) in Japan, de Litardière (1922) in France, Afzelius (1924) in Sweden, Sokolovskaya and Strelkova (1938, 1940 and 1941) in Russia and Rutland (1941) in England give chromosome numbers for various species of Senecio, but, unfortunately, issues of most of the relevant publications are unobtainable in South Africa. Although photostat copies of the more extensive works were ordered nearly a year before the time of writing and enquired about in subsequent correspondence, they have still not arrived here owing to the abnormal conditions prevailing throughout the world. There is, therefore, no way of comparing either the methods used in the present investigation with those used by earlier workers or the morphology and behaviour of the chromosomes of *Senecio isatideus* with those of other Senecio species already investigated.

Without further special study, it would be impossible to decide on the number of genomes, each consisting of several chromosomes differing in form and function but acting together as a complete and harmonious system, making up the chromosome complement. The course of synapsis during prophase I was not traced, but the anaphasic distribution of the chromosomes showed the unfailing regularity which characteristically follows normal synapsis. However, it should not be forgotten that the probable absence of multivalents might also have been the result of the combination of genomes, each of which was present only twice, or of a close genic similarity in the chromosome sets. In the light of current opinion, the restricted habitat might weigh against the possibility of the species being tetraploid because it has been found that, on account of physiological differences between diploids and tetraploids, the latter are often able to grow well in a wider range of habitats than the related diploids. As only one pair of chromosomes of each type was present in the root tip metaphase (see Fig. 1), the species is probably not autotetraploid, if indeed it is tetraploid at all.

M. Navashin (this reference could not be traced) is reported to have found that, as a general rule, the chromosomes tend to spread further away from one another as the turgor of the cell increases. Of the times of soaking at room temperature prior to fixation tried in the present investigation (see p. 111), 3 hours gave the best results.

The first record of the effects of cold treatment on chromosomes was published in 1931 (Delaunay, 1931). More recently, conflicting reports have been made by other workers in this field, and, in particular, Resende, de Lemos-Pereira and Cabral (1944) have attacked, with considerable rancour, the views held by Darlington and La Cour (1940). A summary of the main findings is included in the following part of the discussion, since, to the best of the writer's knowledge, they have not yet been reviewed. The results of observations parallel to those made in the present investigations are stated but no definite correlations are implied, as only a very limited amount of work was done on this aspect of the problem studied.

Delaunay (1931) discovered that keeping Crepis seedlings in finely chipped ice or snow for 24 hours caused complete straightening of the chromosomes with a diminution in length of 50 per cent. Darlington and La Cour (1940) found that, at a temperature at which mitosis stopped in some species, it continues in others but with varying degrees of abnormality, including "differential reactivity" and suppression of the spindle. The spindle is suppressed at marginal temperatures which produce cell anaesthesia without chromosome anaesthesia and this results in "superspiralisation" and "supercontraction" of the chromosomes. Resende, de Lemos-Pereira and Cabral (1944) found that the range of temperature over which the chromosomes decreased in length without showing signs of pathological mitosis was as limited as 1 or 2° C. and differed in each of the three species they studied. They maintain that there is an upper as well as a lower range of temperature over which the

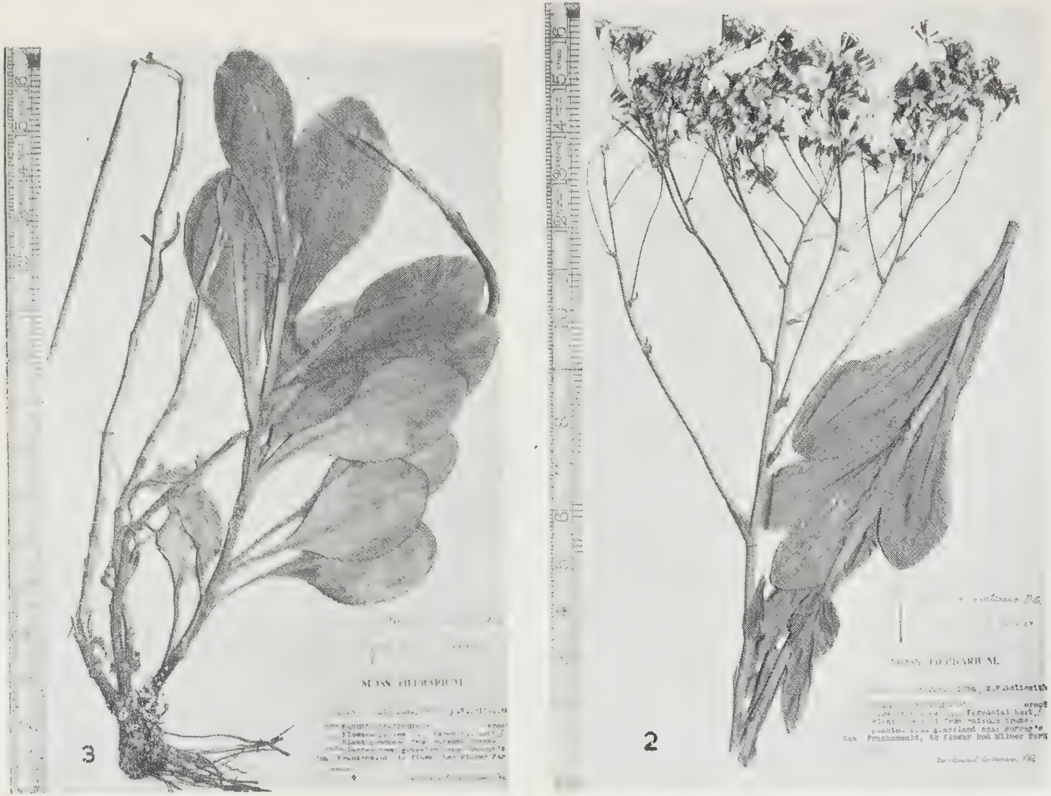

PLATE XIII.
Herbarium sheets of *Senecio isatideus*.

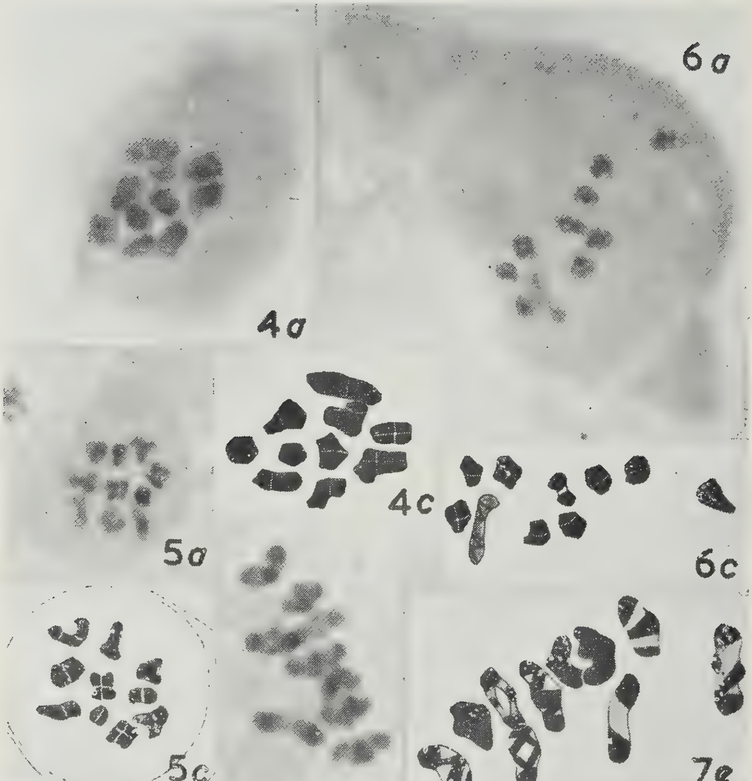

Plate XIV.
(For explanation see opposite).

chromosomes contract. These writers are most critical of the " differential reactivity " described by Darlington and La Cour (1940). Hill and Myers (1945) noted that soaking in water at 2° C. contracted the elongated, overlapping chromosomes of *Dactylis glomerata* and two other forage grasses, facilitating the counting of the chromosomes. Warmke (1946) obtained shortened chromosomes with preservation of morphological details in many different grasses by soaking at 0° C.

2° C. was chosen as the temperature for cold soaking in the present investigation on account of the favourable results reported by Hill and Myers (1945) for three forage grasses receiving pretreatment at this temperature. In light of the findings of Darlington and La Cour (1940) and Warmke (1946) the disappearance of the constrictions (see p. 120) might seem unexpected. However, Resende, de Lemos-Pereira and Cabral (1944) assert that constrictions vary in chromaticity with temperature and become inconspicuous as they approach a completely " pachychromatic " state. (Constrictions with a chromaticity similar to that of the main body of the chromosomes are designated " pachychromatic " by these authors.) They pointed out that this variation was not correlated with the degree of contraction, declaring that they had seen the constrictions at their clearest in much contracted chromosomes. Hill and Myers (1945) found 24 hours the optimum time of soaking at 2° C. for three forage

PLATE XIV.

FIGS. 4—14. Squash acetocarmin.

4*a*. Photograph of a temporary preparation showing polar view of metaphase I. 1900x. Enlargement.
4*c*. Diagram of above. 2300x.
5*a*. Photograph of a permanent preparation showing polar view of metaphase I. Enlargement. 1900x.
5*c*. Diagram of above. 2300x.
6*a*. Photograph of a permanent preparation showing polar view of metaphase I. Enlargement. 1900x.
6*c*. Diagram of above. 2300x.
7*a*. Photograph of a permanent preparation showing equatorial view of metaphase I. Enlargement. 1900x.
7*c*. Diagram of above. 2300x.
8. Diagram of polar view of metaphase I drawn from a temporary preparation. 2300x.
9. Diagram of polar view of metaphase I drawn from a temporary preparation. 2300x.
10. Diagram of polar view of metaphase I drawn from a permanent preparation. 2300x.
12. Diagram of polar view of root tip metaphase after soaking at 2° C. for 6 hours prior to fixation with S. Navashin fixative. 2300x.
13. Diagram of polar view of root tip metaphase after soaking at room temperature for 3 hours prior to fixation with Stockholm—Navashin fixative. 2300x.
14. Diagram of polar view of root tip metaphase after soaking at 2° C. for 14 hours prior to fixation with Lewitsky fixative for big chromosomes with 4 per cent. formaldehyde. 2300x.

grasses. Warmke (1946), on the other hand, found $1\frac{1}{2}$ hours at 0° C. the optimum for many different grasses. In the present study good results were obtained after 14 hours at 2° C. Darlington and La Cour (1940) would attribute the increase in the diameter of the chromosomes with cold treatment (see p. 120) to suppression of the spindle in the absence of which the chromosomes continued to spiralise after the disappearance of the nuclear membrane, presumably developing major spirals. Resende, de Lemos-Pereira and Cabral (1944), on the contrary would attribute the increase in the diameter of the chromosomes neither to an increase in the diameter of the spiral nor to the formation of a greater matrical mass than usual, but to the condensation of the normal matrix around a smaller extension of the chromonemata.

From a consideration of relationships within the Compositae, Small (1919) concludes that the chromosome number is of some value in tracing the phylogeny of the genera of this family. His main thesis, founded on vegetative and floral morphology and physiological and cytological studies, is that Senecio was the first genus of the Compositae to come into existence, originating somewhere between the forests of the Amazon and the heights of the Andes, and that it has directly or indirectly given rise to all the other genera of the family. If Small (1919) is correct, chromosome studies on Senecio and genera closely allied to it would give further evidence of how evolution has progressed within the Compositae. Small (1919) states that his data indicate the 5, 10, 15, 20, 30, 60 series of chromosome numbers in the tribe Senecioneae as the primitive one, with the basic or original haploid number 5 occurring in the genus Senecio. Wanscher (1934) also considers that the basic number of Senecio is 5, reaching this conclusion from an inspection of Tischler's (1927, 1937 and 1938) lists of chromosome numbers. Darlington and Ammal (1945) consider that 5, 9, 12 and 30 are the basic numbers of the tribe Senecioneae and that 10, 11, 12 and 23 are the basic numbers of the genus Senecio. It may be mentioned that some of the species listed by Darlington and Ammal (1945) under " Senecio (Cineraria) " were at one time placed under the genus Cineraria. They were subsequently transferred to Senecio and are evidently still regarded as species of the latter genus by these writers. For the basic numbers of Senecio, Darlington and Ammal (1945) had reference to the chromosome numbers given by Sokolovskaya (1938, 1940 and 1941) as well as to Tischler's (1927, 1937 and 1938) lists. In spite of Afzelius' (1924) record of 5 chromosomes for *Senecio discifolius* which Tischler (1927) included in his lists, Darlington and Ammal (1945) apparently reject 5 as a basic number for the genus, though they do not give their reason for doing so. *Senecio isatideus* would thus have either twice one of the basic numbers, namely twice 5, according to the opinion held by Small (1919) and Wanscher (1934) or one of the basic numbers itself, namely, 10, according to the opinion held by Darlington and Ammal (1945). In the former case, the species would be of tetraploid derivation and probably of allotetraploid derivation at that, since, as has already been pointed out (see p. 120), only one pair of chromosomes of each type was present in the root tip metaphase (see Fig. 1). In any case, *Senecio isatideus* can be added to the list of those species whose chromosome number is a multiple of 5, and, further, to the list of species—which takes in by far the greater number of Senecio species yet studied—whose chromosome number is an even multiple of 5, that is 10 and multiples of 10.

With the aid of the distinctive karyotype described in the present report, it may be possible to decide on the number of genomes composing the somatic chromosome complement in other species of Senecio and to determine their relationship to *Senecio isatideus.*

For the past thirty years, cytologists and taxonomists have been discussing the bearing of chromosome variation on classification in particular groups and, during the latter half of this period, it has been generally acknowledged that a simple determination of the chromosome numbers in plants may be of great importance for taxonomy and ecology. It has been found that differences in chromosome number, morphology and behaviour and variations in characters sometimes correlated with these differences are often useful in classifying varieties and related species. In view of these facts, the undeviating haploid count of 10, further borne out by the diploid count of 20, the constant and characteristic form of the metaphase chromosomes in the root tips by which individual chromosomes could be identified, and the repeated observation of normal anaphasic separation in the pollen mother cells in the present study of *Senecio isatideus* may well assume considerable significance when the cytotaxonomy of other species of Senecio is investigated, and the true natural relationships with their basis in community of origin are discovered.

5. SUMMARY.

In a study on some aspects of the cytology of *Senecio isatideus* DC., a species that is poisonous to grazing animals, temporary and permanent acetocarmine squash preparations of the first and second metaphases of meiosis were photographed and drawn. A haploid number of 10 was recorded.

The somatic chromosomes were studied in sections of root tips fixed in a variety of ways and stained with haematoxylin. A diploid number of 20 was recorded.

The karyotype was drawn after fixation with Navashin and Lewitsky fixatives and the idiogram was worked out. Soaking at room temperature for 3 hours improved the spreading of the chromosomes at the metaphase. Soaking at 2° C. for 14 hours shortened and straightened the chromosomes, increased their diameter and improved their spreading. The constrictions were no longer visible following this treatment.

Stockholm fixative gave the most satisfactory results for general purposes, combining good spreading with preservation of some of the morphological details.

To the best of the writers' knowledge, the present account is the first record of any cytological investigation of a South African species of Senecio.

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